2016 Protocols

Fecal DNA Concentration

Adapted from MoBio's PowerFecal Kit

Purpose:
To extract environmental DNA from fecal samples. This extraction will purify genomic DNA from prokaryotic and eukaryotic cells found in the fecal sample.

Required Materials:
- MoBio PowerFECAL Kit
- Fecal Sample for DNA Extraction
- Disposable Serological Pipette's (or other disposable plastic-ware)
- 70% Ethanol in a Squeeze Bottle
- Weigh Scale
- Vortex Mixer
- Benchtop Centrifuge
- Heat Block or Water Bath at 65˚C

Procedure:
1. Prepare working space and materials in advance. Label PowerFecal column, a dry bead tube and 4 collection tubes appropriately.
2. Weigh 0.25g of fecal sample into the dry bead tube. Use a disposable serological pipette to collect the fecal sample (any other disposable plastic-ware will do here)
    a. Samples with high lipid, polysaccharide or protein content (bird feces for example) may not purify well if too much fecal sample is used. It is recommended to use less fecal sample in these cases.
3. Add 750µL of PowerFecal bead solution to the dry bead tube. Vortex mix briefly.
4. Add 60µL of PowerFecal solution C1 to the dry bead tube and incubate at 65˚C for 10 minutes
    a. If solution C1 has precipitated, incubate at 60˚C until precipitate is gone prior to use.
5. Secure the bead tube horizontally onto a vortex mixer (with MoBio tube holder or tape) and vortex mix at max speed for 10 minutes.
6. Centrifuge for 1 minute at 13,000 x g.
7. Transfer supernatant to a clean collection tube, expecting 400µL – 500µL. (Be careful to avoid the pellet)
8. Add 250µL of PowerFecal Solution C2 and incubate the tube for 5 minutes at 4˚C.
9. Centrifuge again for 1 min at 13,000 *x g*.
10. Transfer up to 600µL of supernatant to clean tube (Avoid pellet again).
11. Add 200µL of PowerFecal Solution C3 and vortex mix. Incubate for 5 minutes at 4˚C.
12. Centrifuge tube at 13,000 *x g* for 1 minute.
13. Transfer supernatant to a clean collection tube. Do not transfer more than 750µL of supernatant at this time (Avoid the pellet)
14. Shake to mix PowerFecal Solution C4. Add 1200µL of Solution C4 and vortex for 5 seconds.

15. Pipette 650µL of supernatant onto Spin Column and centrifuge at 13,000 *x g* for 1 minute. Discard flow-through and repeat until all supernatant has run through the spin column.
16. Add 500µL of PowerFecal Solution C5 to the spin column and centrifuge for 1 mint at 13,000 *x g*.
17. Discard flow-through.
18. Centrifuge again for 1 minute at 13,000 *x g* to remove any residual Solution C5.
19. Place the spin column into a clean collection tube. Be careful not to splash any flow-through from previous step onto the spin column.
20. Add up to 100 µL of PowerFecal Solution C6 to the center of the spin column.
    a. DNase free water can be used as an elution buffer in replacement of Solution C6.
    b. Less Solution C6 can be used for a high concentration end product. Do not use less than 50µL of elution buffer.
21. Centrifuge for 1 minute at 13,000 *x g*.
22. Sterilize all surfaces after performing Fecal DNA extraction, including the scale, with 70% ethanol. Allow prolonged surface contact before wiping up.

The final collection tube now contains pure DNA from the fecal sample. Sample can be stored at -20˚C to -80˚C. See the DNA Concentration protocol for tips on how to concentrate the DNA sample obtained here.